29 DESIPRAMINE INHIBITS THE NICOTINE-INDUCED EXOCYTOSIS IN RAT PHAEOCHROMOCYTOMA CELLS (PC12)

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Already in 1970, Su and Bevan (J Pharmacol Exp Ther 175: 533-540) reported the tricyclic antidepressant desipramine to inhibit the nicotine-induced release of noradrenaline from adrenergic neurons. However, the mechanism of inhibition has not yet been clarified. We investigated the effect of desipramine on the exocytotic release of ^{3}H-noradrenaline in a clonal cell line (PC12) which expresses many properties characteristic of adrenergic neurones.

The PC12 cells were grown in surface culture on plastic dishes coated with collagen. Prior to the experiment, the cells were differentiated by nerve growth factor (200 µg/l for 3 days). The cells were loaded with ^{3}H-noradrenaline (10 nmol/l) for 60 min. After a wash-out period of 30 min the release of ^{3}H-noradrenaline into the incubation medium was stimulated by various methods. The fraction of ^{3}H-noradrenaline (determined after column chromatography) which appeared in the incubation medium during 1 min of stimulation was measured. (1) Depolarization of the cells by potassium (56 mmol/l) released 3.3 ± 0.2 % (n=11) of the intracellular ^{3}H-noradrenaline, (2) the calcium-ionophore A23187 (10 µmol/l) released 1.8 ± 0.2 % (n=7) and (3) nicotine (100 µmol) released 6.0 ± 0.4 % (n=29). Irrespective of the method by which release of ^{3}H-noradrenaline was induced, the release of ^{3}H-noradrenaline absolutely depended on the presence of extracellular calcium. The nicotine-induced release was markedly inhibited by 30 µmol/l hexamethonium but not by 0.5 µmol/l atropine. In some experiments, the release of neuropeptide Y (NPY) - an indicator of exocytosis - was measured by radioimmunoassay. The fraction of NPY which has been released by depolarization (56 mmol/l potassium) and by nicotine (100 µmol/l) corresponded to the release of ^{3}H-noradrenaline. Thus, clonal PC12 cells are a useful model to study exocytosis elicted by the stimulation of nicotinic acetylcholine receptors.

Desipramine potently inhibited the nicotine-induced release of ^{3}H-noradrenaline, the IC_{50} being as low as 61 nmol/l (95% confidence interval: 35, 107; n=6). At 1 µmol/l, desipramine inhibited the nicotine-induced release of NPY and the nicotine-induced rise of intracellular calcium by 97 ± 2 % (n=3) and 89 ± 4 % (n=8), respectively. Intracellular calcium was measured fluorimetrically by the fura-2 method. On the other hand, desipramine was a far less potent inhibitor when the release of ^{3}H-noradrenaline was elicted more directely by depolarization (56 mmol/l potassium) or by a calcium-ionophore (10 µmol/l A23718), both IC_{50}'s being above 3 µmol/l. The results suggest, that desipramine inhibits nicotine-induced exocytosis at an early step in the sequence of events which eventually leads to exocytosis. Most probably, desipramine blocks the nicotinic acetylcholine receptor or the cation channel which is closely associated with that type of receptor.

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